

APPLICANT (S): PLESTED, Joyce S. et al.
SERIAL NO.: 10/089,583
FILED: July 11, 2002
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In the Specification:

Please amend page 1, line 2, as follows:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a National Stage Application under 371 of PCT/GB00/03758, filed 10/02/00, now abandoned, which claims priority of U.S. Provisional Application 60/196,305, filed 4/12/00 and U.S. Provisional Application 60/156,940, filed 09/30/99, which are hereby incorporated by reference.

Please insert the following on page 18, line 27:

Brief Description of the Drawings

Please amend page 18, fourth full paragraph, as follows:

Figures 1a-1b illustrate[[s]] the LPS structure of various *Neisseria meningitidis* immunotypes;

Please amend page 19, line 4, as follows:

Figures 3a-3c illustrate[[s]] molecular models of the calculated (MMC) lowest energy states of the core oligosaccharide from *galE* mutants of L3, L4, and L8 dephosphorylated;

Please amend page 19, line 10, as follows:

Figures 5a-5d illustrate[[s]] confocal immunofluorescence microscopy of *Neisseria meningitidis* organisms strain MC58 adherent to HUVECs;

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Please amend page 19, line 13, as follows:

Figures 6a-6b illustrate[[s]] silver stained tricine gels of LPS preparations from group B strains not reactive with B5;

Please amend page 19, line 16, as follows:

Figures 7a-7d illustrate[[s]] accessibility of the LPS epitope to A4 in *Neisseria meningitidis* whole cells;

Please amend page 20, line 23, as follows:

Figures 15a-15b illustrate[[s]] a FACS profile comparing surface labeling of live *Neisseria meningitidis* MC58 and *galE* mutant (5 x 10⁸ org/ml) with MAb (culture supernatant 1:50) detected using anti-mouse IgG (FITC labeled).

Please amend page 46, first full paragraph, as follows:

Figures 1a-1b

Please amend page 46, fifth full paragraph, as follows:

Figures 3a-3c

Please amend page 47, third full paragraph, as follows:

Figures 5[[.]]a[[.]]-5d

Please amend page 47, fifth full paragraph, as follows:

Figures 6a-6b

Please amend page 50, ninth full paragraph, as follows:

See Figures 7a-7d.

Please amend page 51, sixth full paragraph, as follows:

See Figures 3a-3c.

Please amend page 59, second full paragraph, as follows:

Figures 15a-15b. FACS profile comparing surface labeling of live *Neisseria meningitidis* MC58 and *galE* mutant (5×10^8 org/ml) with MAb (culture supernatant 1:50) detected using anti-mouse IgG (FITC labeled).

Please amend page 46, line 5, as follows:

Representation of the structure of meningococcal LPS oligosaccharides of immunotypes L1 to L9. Immunotypes are indicated to the extreme left. The vertical line marks the junction between the inner core structures to the right and outer core structures to the left. The epitope recognized by MAb B5 is ~~indicated in boldface~~ underlined (MAb B5 positive). Arabic numerals indicate the linkage between sugars or amino sugars. Alpha and beta indicate the carbon 1 linkage at the nonreducing end of the sugar. Genes for incorporating each of the key sugars or amino sugars into the LPS oligosaccharide in the biosynthetic pathway are indicated with arrows indicating where in the pathway the gene product is required. Abbreviations: Kdo, 2-keto-2-deoxyoctulosonic acid; Gal, galactose; GlcNAc, N-acetylglucosamine; Glu, glucose; Hep, heptose. Immunotype L5 has no PEtn on the second heptose. The gene that adds the glucose to the second heptose (lgtG) is phase variable.

Please amend page 27, third full paragraph, as follows:

A solid-phase indirect ELISA with purified LPS was used to determine the binding specificities of MAbs. Nunc Maxisorp® plates were coated overnight with 1.0 µg of purified LPS per well derived from wild-type and mutants. LPS (10 µg/ml) was diluted in 0.05 M carbonate buffer containing 0.02 M MgCl₂ (pH 9.8). Nonspecific binding sites were blocked for 1 h with 1% BSA-PBS (Sigma) and washed three times with PBS-Tween® 20 (0.05% [vol/vol]; PBS-T). Plates were incubated for 1 h with MAb B5 culture supernatant and washed three times in PBS-T. Primary antibody was detected with anti-mouse IgG-alkaline phosphatase (Sigma and Cedarlane Laboratories, Ltd.) incubated for 1 h, washed three times in PBS-T, and detected with p-nitrophenyl phosphate alkaline phosphatase substrate system

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(Sigma and Kirkegaard & Perry Laboratories). The reaction was stopped after 1 h with 50 μ l of 3 M NaOH, and the absorbance ($A_{405-410}$) was determined (Dynatech ELA plate reader

Please amend page 28, third full paragraph, as follows:

Whole cell (WC) ELISA was performed by using heat-inactivated lysates of *N. meningitidis* organisms as described previously (Abdillahi, H., and J. T. Poolman. 1988. J. Med. Microbiol. 26:177-180). Nunc Maxisorp® 96-well plates were coated with 100 μ l of bacterial suspension (optical density [OD] of 0.1 at A620) overnight at 37°C and blocked with 1% BSA-PBS; an identical protocol was followed as for LPS ELISA.

Please amend page 53, first full paragraph, as follows:

Briefly, fluorescently labeled ethanol fixed *Neisseria meningitidis* MC58 or *galE* mutant or beads coated with purified *galE* LPS (10ug/ml) were opsonized with MAb B5 and human complement source diluted in final buffer for 10mins/37C/ 500rpm in microtitre plate. Then human peripheral blood polymorphonuclear cells (MPMs) prepared from heparinised donor blood were diluted in final buffer and added to each well (1×10^7 cells/ml) and incubated for a further 10mins/37C/ 500rpm. Reaction mixture was stopped on ice by addition of 150 ml PBS-EDTA and added to FACS tube containing 50 μ l Trypan Blue®. Mixture was mixed and 10,000 lymphocytes were analyzed on FACScan and Cellquest software. PMSs were analyzed by FSC vs appropriate channels to determine % uptake of fluorescent bacteria by granulocytes and monocytes (% OP activity).

Please amend page 54, second full paragraph, as follows:

(5) Affinity purified MAb B5. Spent culture supernatant from MAb B5 was purified on Protein A-sepharose® column and eluted with Glycine pH 4.5, neutralised with Tris-HCl pH 9.0. Fractions were tested for reactivity on LPS ELISA, pooled and concentrated using Amicon®-filter. Purity was determined by SDS-PAGE gel and protein concentration was determined by OD and protein assay.

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AMENDMENTS TO THE DRAWINGS

The attached sheet of drawings includes changes to Fig. 1 and 15. These sheets, which include Fig. 1 and 15, replace the original sheets including Fig. 1 and 15.

Attachment: Replacement Sheet